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<p>Since visual inputs to the light-entrainable circadian pacemaker in the suprachiasmatic nucleus (SCN) presumably trigger a cascade of cellular events locally, studies were conducted during the current budget year to examine the photic regulation of cellular activity within the SCN. Experiments examined the effect of optic nerve stimulation on peptide release and mRNA levels expressed by perfused SCN explants. Emphasis was placed on studying populations of SCN neurons containing vasoactive intestinal polypeptide (VIP) or vasopressin (VP) because sensitive antibodies for radioimmunoassay and transcripts for analyzing mRNAs that encode these peptides are readily available.</p> <p>Irrespective of the time of treatment, optic nerve stimulation evoked acute increases in VP release, such that VP output during stimulation was 2-4 times greater than that observed in control explants. Within 2-4 hr after stimulation, VP release declined to pre-treatment levels. The effect of optic nerve stimulation on VP mRNA content in SCN explants was dependent on the time of treatment; VP mRNA was increased by 25-40% following stimulation during the subjective day, but was unchanged after stimulation during the subjective night. Release of VIP and VP mRNA content expressed by SCN explants was unaffected by stimulation, irrespective of the time of treatment. These results suggest that VP neurons in the SCN, although devoid of retinal innervation, may be responsive to photic stimulation. Analysis of the effect optic nerve stimulation at different circadian times and using different parameters will be conducted to complement these observations, but the present approach may provide a basis for examining the light responsiveness of different cell types within the SCN.</p>					
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Objectives

The objective of this research project is to examine the molecular and cellular events that characterize the secondary transduction of photic signals by neurons of the suprachiasmatic nucleus (SCN). Experiments will utilize organ-cultured explants of the SCN because this approach has provided a unique model for analyzing both the acute and phase-shifting responses of SCN neurons to various neurochemical and physiological signals. The research project entails two series of experiments in which this *in vitro* approach will be coupled with physiological and molecular analyses to systematically examine the influence of photic signals on peptidergic systems in the SCN. In the first project, experiments will focus on the secretion of specific peptides by cells in the SCN. The second project will parallel this analysis by evaluating the content of specific mRNAs encoding for these peptides in SCN neurons. Emphasis in the experiments has been placed on evaluating peptide secretion and mRNA expression for four groups of peptidergic neurons that are intrinsic to the SCN, receive visual input and/or maintain extensive local connections, namely vasopressin (VP), somatostatin, vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI). The specific objectives of this project are:

- 1) To first characterize the release of somatostatin, VIP and PHI and the content of mRNAs encoding for vasopressin and these peptides expressed by the SCN explant system.
- 2) To evaluate the effects of acute stimulation of the optic nerves on the secretory activity of peptidergic neurons and on the content of these peptide mRNAs in the SCN explant system.

The parallel analysis of the effects of photic inputs on peptide release and the levels of specific mRNAs encoding for these peptides in the SCN may provide an opportunity to determine whether the coordinated regulation of peptide synthesis and release occurs in the SCN, like in other systems using peptides as chemical signals. Since the retinohypothalamic tract represents the only viable pathway capable of relaying photic signals to the explanted SCN, this approach will also provide a basis for distinguishing its specific role in mediating the photic regulation of SCN cellular activity from that of the visual pathway emanating from the ventral lateral geniculate nucleus and intergeniculate leaflet of the thalamus. In addition, these experiments may contribute to our understanding of the molecular basis for the photic control of SCN cellular activity and function, and by providing a gene marker(s) that underlie the resetting of the circadian system by light, may ultimately be useful in the treatment, diagnosis and understanding of health problems related to occupational and travel schedules that have been associated with a disruption of circadian timekeeping.

Current Status of Research Project

Since the neural inputs relaying photic signals to the SCN presumably trigger a cascade of cellular events locally before this information is conveyed to effector systems, studies were conducted during the current budget year to examine the photic regulation of cellular activity within the SCN. Experiments were designed to examine the effect of optic nerve stimulation on peptide release and mRNA content expressed by perfused SCN explants. Emphasis during this period was placed on studying the distinct populations of SCN neurons containing VP or VIP because sensitive antibodies for radioimmunoassay and transcripts for analyzing mRNAs that encode these peptides are readily available.

Suprachiasmatic explants, consisting of the bilateral SCN, their rostral projections to the organum vasculosum of the lamina terminalis and the underlying optic chiasm, were dissected from the hypothalami of male Sprague-Dawley rats. Explants were placed in individual chambers



with the optic nerves housed positioned in a modified cuff electrode. The chambers, in turn, were placed in small volume cell culture system and medium was perfused over the explants at a rate of ≈ 0.8 ml/hr.

Electrical impulses (duration = 0.15 msec.; amplitude = 10V; rate = 2 Hz) were applied for 10-15 sec to both optic nerves at different times during the circadian cycle. To evaluate any changes in peptide release or mRNA content induced by optic nerve stimulation, simultaneous analysis was conducted on control explants subjected to either electrical stimulation of the surrounding medium or no electrical stimulus. The pattern of peptide release before, during and after these control and experimental manipulations was analyzed by collecting serial samples of the perfusate at 2-hr intervals for 1-2 days. Peptide concentration in the culture medium was determined by radioimmunoassay. In separate experiments, analysis of peptide mRNA content in SCN explants was conducted under similar conditions, except that all explants were removed from the culture system and frozen in liquid nitrogen within 60-90 min after control and experimental manipulations. In addition, complementary information on peptide release was collected until termination of these experiments as indicated above. A solution hybridization/RNA protection assay was used to quantitate peptide mRNA content in control and stimulated explants. Tissue from 2 control or 2 experimental explants was homogenized together and total RNA was extracted using a protocol described by Sherman and Watson. Extracted RNA samples were hybridized with ^{32}P -labeled cRNA probes synthesized from full-length constructs. Following completion of the hybridization reaction, nonhybridized mRNA was digested with RNase A and RNase T₁. Protected cRNA:mRNA fragments were fractionated on agarose gels.

Irrespective of whether electrical stimulation of the optic nerves was administered during the subjective day or during the subjective night, this treatment consistently induced acute increases in VP release (Figure 1). In fact, VP release over the 2-hour sampling interval coinciding with the period of optic nerve stimulation was 2-4 times greater than that observed in control explants over the same interval (Figure 2). Following optic nerve stimulation, VP release from SCN explants returned to pre-stimulation or baseline levels within 2-4 hours. Optic nerve stimulation also had a stimulatory effect on VP mRNA content in SCN explants, but this effect was dependent on the time of treatment (Figure 3). Following optic nerve stimulation during the subjective day, VP mRNA content was significantly greater than that expressed by control explants, but was unchanged relative to control explants following stimulation during the subjective night (Figure 2). The increment in VP mRNA content following optic nerve stimulation during the subjective day ranged from 25-40% by comparison with the content observed in control explants.

Requisite to analysis of the effect of optic nerve stimulation on VIP release, preliminary experiments were conducted to characterize the temporal pattern of VIP secretion from SCN explants. Similar to the temporal pattern for VP secretion, VIP was released in a circadian fashion from most SCN explants (Figure 4). The circadian pattern of VIP release from SCN explants was characterized by an increase in VIP output near the middle of the subjective night, peak levels of release during the early subjective day and a precipitous decline in VIP release to minimal levels that persisted throughout the remainder of the circadian cycle. VIP release during the peak of the rhythm was 3 to 6 times greater than that during the nadir. Among individual explants, the patterns of VIP release were homogeneous with regard to their circadian waveform. Analysis of VIP release was only conducted for a maximum of 2 days, but the circadian rhythms persisted with little sign of damping over this period of time.

Due in part to technical problems, the effects of optic nerve stimulation on VIP neurons in SCN explants were equivocal. Stimulation of the optic nerves during the early subjective night failed to evoke any notable change in VIP release from SCN explants (Figure 4 and 5), while

analysis of VIP output in response to stimulation during the subjective day was compromised by problems with the radioimmunoassay. The VIP mRNA content found in SCN explants was also unaffected by optic nerve stimulation (Figure 5), irrespective of whether this treatment occurred during the subjective day or night. While these observations suggest that VIP neurons may be insensitive to visual stimulation, additional studies examining the effect of optic nerve stimulation at different times and using different parameters will be conducted during the next year to complement these observations and further investigate the visual responsiveness of VIP neurons in SCN explants.

Experimentation during the next budget year is expected to proceed as originally proposed. Experiments will involve further evaluation of the effect of optic nerve stimulation on peptide release and mRNA content expressed by SCN explants, with continued emphasis on the VP and VIP components within the nucleus. In addition, studies will be initiated in attempt to generate similar analyses for somatostatin and PHI since neurons containing these peptides also represent prominent components of the SCN that receive retinal input and/or maintain extensive local connections. Preliminary studies have already been conducted using transcripts for the somatostatin and PHI genes to demonstrate that the mRNAs encoding for these peptides are detectable in SCN explants. In addition, several antibodies have been tested for use in radioimmunoassay of somatostatin and PHI levels in media samples. Since the sensitivity of these antibodies appears to be inadequate for reliably detecting the levels of these peptides that SCN explants release into the culture medium, attempt to manipulate assay conditions so as to improve the sensitivity of these antibodies and to test new antibodies for the same purpose.

Professional Personnel

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Publications and Interactions

Earnest, D.J., Gallagher, M., Yagil, C. and Sladek, C.D. (1990) Effect of optic nerve stimulation on peptide release and mRNA levels expressed by cultured suprachiasmatic explants. Soc. for Neuroscience Abstr. 16:771.

1991 Winter Brain Conference (Vail, CO); Panel on "Current status of circadian rhythm regulation in mammals".

FIGURES

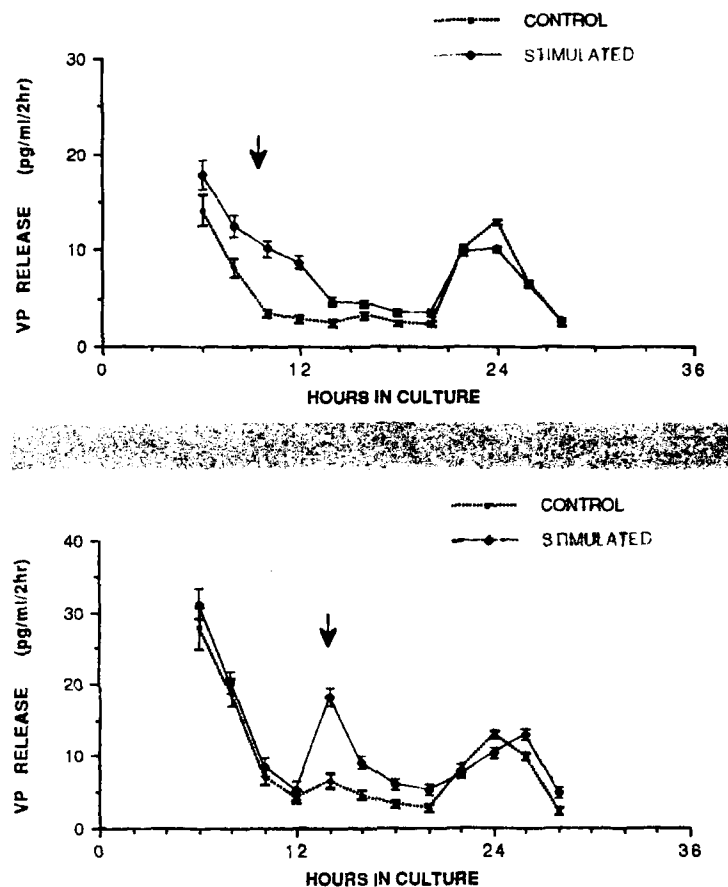


FIGURE 1. Temporal profiles of VP release from rat SCN explants subjected to no treatment (**CONTROL**; N=6) or electrical stimulation of the optic nerves (**STIMULATED**; N=12) during the subjective day (top) or during the subjective night (bottom). The actual times of treatment are indicated by the arrows above the VP profiles. Closed symbols denote determinations of the mean concentration of VP released into the medium over two-hour sampling intervals.

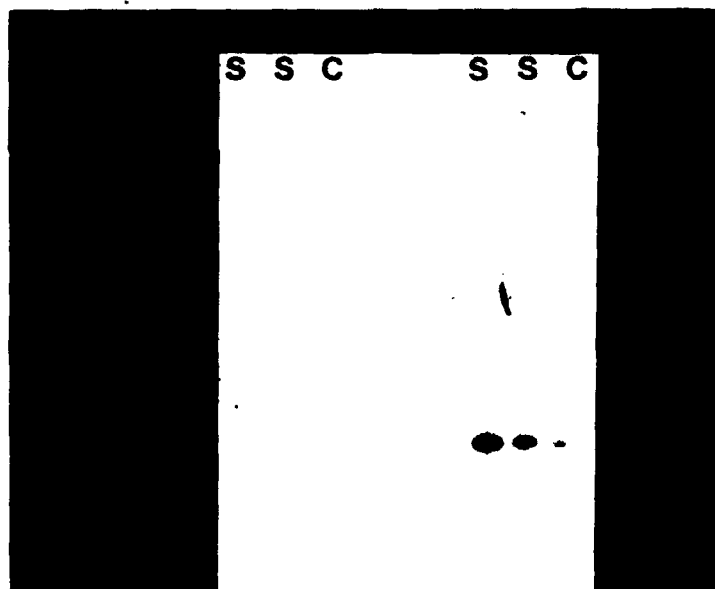


FIGURE 2. Analysis of the effect of optic nerve stimulation on VP mRNA content of SCN explants at different times during the circadian cycle. 1, The VP cRNA:mRNA protected fragments from untreated control explants (C) or explants subjected to optic nerve stimulation (S) during the subjective day. 2, Protected fragments from untreated control explants (C) or explants subjected to stimulation of the optic nerves (S) during the subjective night. Autoradiograms depict the fractionation of protected fragments after solution hybridization of single-stranded VP-specific ^{32}P -cRNA probe to total RNA extracted from SCN tissue. Each lane represents the total nucleic acids from the pooled SCN of 2 rats. The specific band sizes of about 700 bases correspond to the expected size of the fragment protected by endogenous VP mRNA.

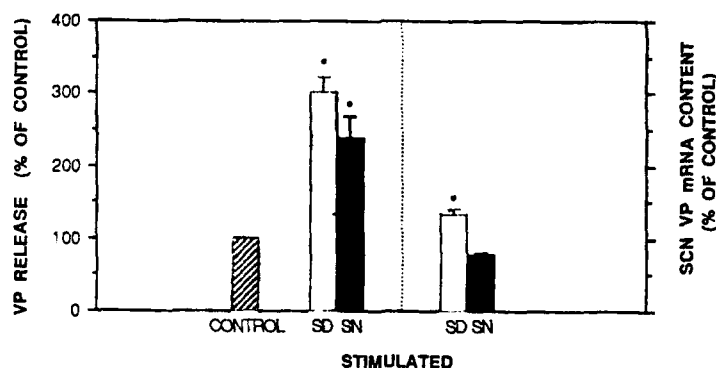


FIGURE 3. Effect of optic nerve stimulation on VP release and VP mRNA content expressed by SCN explants. VP release during optic nerve stimulation and VP mRNA content following stimulation (STIMULATED) are expressed as a percentage (mean \pm SEM) of the values obtained from untreated control explants (CONTROL). Explants were subjected to optic nerve stimulation either during the subjective day (SD) or the subjective night (SN).

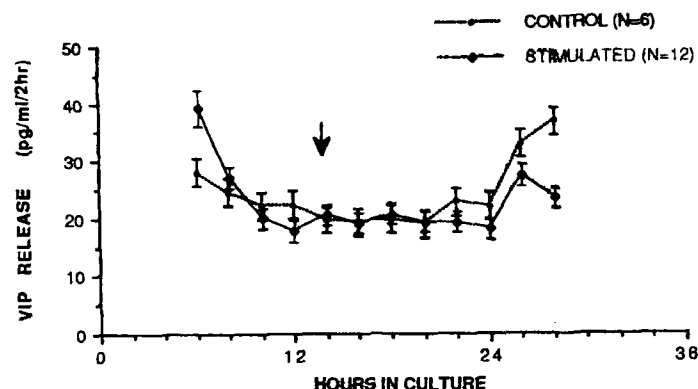


FIGURE 4. Temporal profiles of VIP release from rat SCN explants subjected to no treatment (**CONTROL**) or electrical stimulation of the optic nerves (**STIMULATED**) during the subjective night. The actual time of treatment is indicated by the arrow above the VIP profiles. Closed symbols denote determinations of the mean concentration of VIP released into the medium over two-hour sampling intervals.

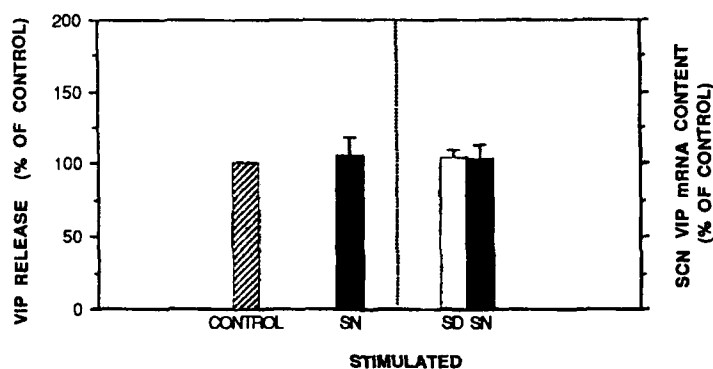


FIGURE 5. Effect of optic nerve stimulation on VIP release and VIP mRNA content expressed by SCN explants. VIP release during optic nerve stimulation and VIP mRNA content following stimulation (**STIMULATED**) are expressed as a percentage (mean \pm SEM) of the values obtained from untreated control explants (**CONTROL**). Explants were subjected to optic nerve stimulation either during the subjective day (**SD**) or the subjective night (**SN**).